ACCELERATED COMMUNICATION

BEST AVAILABLE COPY

Paclitaxel (Taxol) Inhibits Protein Isoprenylation and Induces Apoptosis in PC-3 Human Prostate Cancer Cells

ROMANO DANESI, WILLIAM D. FIGG, EDDIE REED, and CHARLES E. MYERS

Division of Hematology/Oncology, University of Virginia, Charlottesville, Virginia 22908 (R.D., C.E.M.), and Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (W.D.F., E.R.)

Received October 24, 1994; Accepted February 24, 1995

SUMMARY

Paclitaxel was examined for its effects on cell survival, internucleosomal DNA fragmentation, and protein isoprenylation in the human prostate cancer cell line PC-3. Treatment of cells with paclitaxel at 5–60 nm for 24 hr resulted in a dose-dependent inhibition of cell viability (IC $_{50}$, 31.2 nm), which was partially prevented by supplementing the cell culture medium with two nonsterol polyisoprenyl compounds, farnesyl-pyrophosphate (-PP) and geranyigeranyl-PP (3 μ m each). Furthermore, agarose gel electrophoresis of DNA extracted from cells treated with paclitaxel (15–60 nm) for 24 hr showed DNA laddering with production of fragments of 18D-base pair multiples, indicating the occurrence of apoptotic cell death. Internucleosomal DNA fragmentation by paclitaxel was also detected by a photometric enzyme immunoassay using antihistone antibodies; if culture

medium was supplemented with farnesyl-PP and geranylgeranyl-PP (3 μ M each), a reduction in mono- and oligoucleosome production was observed. The post-translational incorporation of metabolites of (RS)-[5-³H]mevalonolactone (100 μ Ci/ml) into prenylated proteins of PC-3 cells was inhibited by paclitaxel at 30 and 60 nM. In addition, the immunoprecipitation of p21ras and p21rap-1 proteins from PC-3 cells exposed to paclitaxel (30 and 60 nM) and labeled with (RS)-[5-³H]mevalonolactone showed a substantial inhibition of the incorporation of farnesyl and geranylgeranyl prenoid groups, respectively, into the aforementioned proteins. These results indicate that the inhibition of protein isoprenylation is a novel component of the complex biochemical effects of the drug and plays an important role in the mechanism of paclitaxel cytotoxicity in PC-3 cells.

Eukaryotic polypeptides that are initially synthesized with the carboxyl-terminal amino acid sequence CAAX, including a variety of signal-transducing proteins such as G proteins and cGMP phosphodiesterases, can be targeted for a series of sequential post-translational modifications (1). This novel processing pathway includes the isoprenylation of the cysteine residue with a C₁₅ farnesyl or C₂₀ geranylgeranyl moiety, followed by proteolysis of the three terminal residues and α -carboxyl methyl esterification of the cysteine residue (2). The isoprenoid farnesyl-PP is a particularly important intermediate in the mevalonate pathway. It is used to synthesize cholesterol (3), and it is also bound covalently to the proteins encoded by the ras oncogenes (4), whose mutated forms are among the most common genetic abnormalities in human cancers (5). In addition, ras-related, low molecular weight G proteins, including the products of the rap-1, rab, and rho

genes, have been shown to be geranylgeranylated (1). Thus, isoprenylation is a critical step for subcellular localization of and acquisition of biological activity by signal-transducing proteins that play a pivotal role in cell growth regulation.

Inhibitors of the enzyme HMG-CoA reductase, such as lovastatin, block the production of mevalonate and its metabolites, including farnesyl-PP and geranylgeranyl-PP, and have been shown to suppress the proliferation of many cell types (6). Inhibition of isoprenoid biosynthesis by lovastatin triggers apoptosis in the human promyelocytic cell line HI_60 (7), an effect that is also produced by paclitaxel in the same cell line (8). Paclitaxel is a terpene compound obtained from the bark of Taxus brevifolia and is characterized by strong affinity for tubulin protein and remarkable antitumor activity in vitro and in vivo (9). Apart from its well known antimicrotubular effect, other pharmacodynamic properties of the drug are still to be examined. In the present study, the effects of paclitaxel on apoptosis and protein prenylation were investigated in the human prostate cancer cell line PC-3.

ABBREVIATIONS: PP, pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; bp, base pair(s); MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2-H-tetrazolium; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

R.D. is from the Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna (Pisa, Italy). Financial support from the Italian Association for Cancer Research (Milano, Italy) to R.D. is gratefully acknowledged.